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Fundamental research questions in subterranean biology

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Running title: Scanning the horizon of subterranean biology

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ABSTRACT

Five decades ago, a landmark paper in *Science* titled *The Cave Environment* heralded caves as ideal natural experimental laboratories in which to develop and address general questions in geology, ecology, biogeography, and evolutionary biology. Although the ‘caves as laboratory’ paradigm has since been advocated by subterranean biologists, there are few examples of studies that successfully translated their results into general principles. The contemporary era of big data, modelling tools, and revolutionary advances in genetics and (meta)genomics provides an opportunity to revisit unresolved questions and challenges, as well as examine promising new avenues of research in subterranean biology. Accordingly, we have developed a roadmap to guide future research endeavours in subterranean biology by adapting a well-established methodology of 'horizon scanning' to identify the highest priority research questions across six subject areas. Based on the expert opinion of 30 scientists from around the globe with complementary expertise and of different academic ages, we assembled an initial list of 258 fundamental questions concentrating on macroecology and microbial ecology, adaptation, evolution, and conservation. Subsequently, through online surveys, 130 subterranean biologists with various backgrounds assisted us in reducing our list to 50 top-priority questions. These research questions are broad in scope and ready to be addressed in the next decade. We believe this exercise will stimulate research towards a deeper understanding of subterranean biology and foster hypothesis-driven studies likely to resonate broadly from the traditional boundaries of this field.

Key words: biospeleology, cave biology, expert opinion, groundwater, horizon scanning, research questions, stygofauna, troglobionts.

CONTENTS

I. INTRODUCTION

In the era of the Internet, social media, and open-access mega-journals, the amount of accessible scientific information is overwhelming (Landhuis, 2016; Wakeling *et al.*, 2016; Fire & Guestrin, 2019; Jarić *et al.*, 2020). It is estimated that more than 50 million peer-reviewed scientific papers exist (Jinha, 2010) and about 1.5 million new articles are published every year (Laurance *et al.*, 2013). To capitalize on the volume of this information and make the most of it (e.g. Ioannidis, 2005; Jeschke *et al.*, 2019), it is becoming increasingly important for scientists to explore effective ways to capture the latest advances in their field or related fields of research. Horizon scanning – i.e. the systematic examination of information to identify emerging issues and opportunities in a given research area – has become a useful tool to summarize and determine research priorities and agendas (Sutherland *et al.*, 2011). The most important questions in ecology (Sutherland *et al.*, 2013; McGill *et al.*, 2019), island biogeography (Patiño *et al.*, 2017), and microbiology (Antwis *et al.*, 2017), the annual identification of emerging issues in global conservation (Sutherland *et al.*, 2020), as well as the 100 articles that every ecologist should read (Courchamp & Bradshaw, 2018), are all instructive examples where horizon scanning has successfully synthesized trends or highlighted the most promising future research avenues.

Fifty years ago, in a landmark *Science* paper titled *The Cave Environment*, Poulson & White (1969) heralded caves as 'natural laboratories', i.e. simplified settings that can be used to understand the principles governing the dynamics of more complex environments. Characterized by stringent environmental constraints and simple communities, subterranean habitats have been regarded as ideal systems for investigating many of the unresolved questions in ecology, biogeography, and evolutionary biology (Juan *et al.*, 2010; Sánchez-Fernández *et al.*, 2018; Mammola, 2019). Scientists have also studied subterranean organisms to understand human diseases such as autism (Yoshizawa *et al.*, 2018), diabetes (Riddle *et al.*, 2018), and cancer (Gatenby, Gillies & Brown, 2011), to investigate the engineering potential of biologically inspired materials (Lepore *et al.*, 2012), and to discover new drugs and pharmaceutical products (Cheeptham *et al.*, 2013). Others have even looked at caves through the lens of astrobiology, showing that the subterranean microbiome might hold clues to life beyond Earth (Northup *et al.*, 2011; Popa *et al.*, 2011).

Although the 'caves as laboratory' paradigm is often advocated by subterranean biologists, examples of studies that have successfully translated their results into general principles remain few in number. Five decades after Poulson & White (1969), subterranean biology is entering a new research era dominated by big data (Zagmajster *et al.*, 2019), modelling tools (Flôres *et al.*, 2013; Mammola & Leroy, 2018), and increasingly cheaper molecular approaches (Pérez-Moreno, Iliffe & Bracken-Grissom, 2016; Lefébure *et al.*, 2017).

Concomitantly, we are facing a global crisis that is negatively impacting subterranean biodiversity (Mammola *et al.*, 2019*b*; Boulton, 2020). Therefore, the time is ripe to review the outstanding challenges faced by this broad-in-scope discipline, as well as promising new research avenues where subterranean-based studies may be helpful in answering general and broadly scoped questions. Because gathering multiple views on such an extensive subject is difficult, we relied on the well-established methodology of horizon scanning to identify 50 fundamental, but unresolved questions in subterranean biology. With this intellectual exercise, we aimed to develop a roadmap that will guide future research endeavours and stimulate hypothesis-driven studies likely to resonate beyond the boundaries of this discipline.

II. HORIZON SCANNING PROTOCOL

(1) Initial list assembly

We used horizon scanning methodology (Sutherland *et al.*, 2011) and adapted the approach developed by Patiño *et al*. (2017) to identify priority research questions in subterranean biology. Survey coordinators (S.M. and P.C.) identified seven subject areas within the subterranean biology discipline (Table 1), namely: (1) Adaptation, (2) Origin and evolution, (3) Community ecology, (4) Macroecology and biogeography, (5) Conservation biology, (6) Microbiology and applied topics, and (7) Other topics. We included the latter subject area to cover additional topics or ideas that departed from the six core subject areas and may have been overlooked. For each subject area, survey coordinators invited a senior researcher (highlighted with asterisks in Table 1) to act as panel coordinator, with the task of establishing an international panel of experts to identify and formulate a set of fundamental questions. Each panel coordinator selected and invited three or four members to join their panel, which included at least one early-career scientist (i.e. a postdoc or researcher with less than 10 years of experience) to obtain a multi-generational perspective on the different topics. Survey coordinators encouraged panel members to consult broadly with colleagues and select additional researchers to join their panels if deemed important in providing complementary expertise. In assembling the panels, our goal was to maximize multidisciplinarity, while ensuring that research interests within the seven panels covered a broad array of geographic areas, model organisms, and networks of international collaborators. Members of each panel identified at least 20 questions that they viewed as fundamental within their subject area and thus likely to advance the field significantly.

In total, we assembled 258 questions, which were screened for duplication or ambiguity by the survey coordinators. In this phase, survey coordinators purged most subterranean-specific jargon from questions and homogenized wording to ensure that all questions were presented in a clear and straightforward manner. Therefore, throughout the survey we operated under the assumption that all questions were characterized by a similar degree of readability (Plavén-Sigray *et al.*, 2017). After the cleaning procedure and removal of duplicate questions, we assembled a final list of 211 survey questions (hereafter 'List #1'). In assembling List #1, we subsumed questions identified by the panel focusing on 'Other topics' into the six main subject areas.

(2) Voting procedure and selection of 50 top-priority questions

We subjected List #1 to an initial round of online voting by all panel members (Survey #1) to select the most voted 20 questions for each of the six subject areas (Fig. 1). Voting was a binary choice, whereby participants scored each question as either of 'major' or 'minor' importance. We randomized question order for each participant. We repeated this voting protocol in all subsequent online surveys. Each panel member voted on all questions irrespective of subject area, although votes by panelists on their subject area were disregarded in the final ranking of Survey #1. As a result, survey coordinators culled List #1 to the 120 most-voted questions (20 questions from each of six subject areas), referred to as List #2, thus balancing the number of questions in subsequent voting rounds.

We then subjected List #2 to online voting (Survey #2) by inviting a broad community of subterranean biologists including *ca.* 170 members of the International Society on Subterranean Biology (ISSB), *ca.* 50 members of the European Cave Organism Network, *ca.* 100 members of the Anchialine mailing list, as well as other working groups and email listservs related to subterranean biology that we could identify (e.g. national biospeleological groups). Note that members of these different groups often overlapped and some of the emails were no longer active. We estimated that Survey #2 reached an upper boundary of between 200 and 250 unique recipients. Of these, 133 recipients completed the online survey.

At the end of Survey #2, we gave participants the opportunity to submit one additional question if they felt this question was missing from List #2. Thus, 25 additional questions were added to the third list of questions (List #3). Questions in List #3 were voted on by all panel members (Survey #3), and then ranked (by percentage of 'major importance' votes per question) together with the 120 questions from List #2. Finally, we selected the highest ranking questions to assemble a list of 50 top-priority questions.

(3) Caveats on interpretation

Some general caveats should be recognized when interpreting the results of any horizon scanning survey (e.g. Sutherland *et al*., 2011, 2013; Seddon *et al*., 2014; Patiño *et al*., 2017). First, the background knowledge and intellectual passions of the experts involved may introduce subjectivity in the formulation of the initial list of topics and questions. Second, subjectivity likely plays a role throughout the voting process, as any voting outcome may be affected by the interests of a particular group of participants. In our case, potential biases in the composition of subterranean biologists sampled may have influenced the final selection of the top-priority questions to an extent difficult to quantify precisely. For example, questions related to microbiology received the lowest share of 'major importance' votes (mean ± SD: 0.69 ± 0.01). It is understood that microbiology topics are not less important or timely, it is simply that microbiologists are probably underrepresented in the subterranean biology community. Also, an imbalance in the expertise of participants may explain the substantial difference in how the highest priority questions were parsed across the six subject areas – from four in 'Community ecology' to 12 in 'Conservation biology'.

To address these potential shortcomings, we adopted four countermeasures. First, we increased the survey audience, by addressing the questionnaire to different groups and associations of subterranean biologists. Second, we diversified the expertise of panel members by including early-stage to mid- and late-career researchers from different disciplines, research laboratories, and geographic areas. Third, we included a seventh panel ('Other topics') specifically to fill the gaps in the initial composition of proposed questions. Indeed, it has been argued that in horizon scanning, the initial division into subject areas may limit lateral thinking (Sutherland *et al.*, 2013). Finally, by allowing voters to suggest additional questions when voting in the survey, we were able to capture the range of priority topics better.

We are confident these practices minimized some of the biases inherent to this study. Importantly, we believe this 50 top-priority survey served to highlight some of the most timely and challenging areas of interest in current and future research, rather than providing a comprehensive synthesis of research needs in modern subterranean biology.

III. SUMMARY OF THE HORIZON SCAN

In Survey #1, the percentage of 'major importance' votes ranged between 89% (top-voted question) and 4% (least-voted question). In the extended online voting (Survey #2), 133 voters participated, of which 71% identified 'subterranean biology' as their primary field of research. Although voters' gender was slightly skewed toward males (76 men *versus* 57 women), deviation from the 1:1 male:female ratio was not significant ($\chi^2 = 2.71$; d.f. = 1; $P = 0.10$), indicating that our sample was not gender-biased. 45% of survey voters were experienced researchers, with an academic age of more than 10 years since they earned their PhD, while 29% were researchers within 10 years from their PhD. PhD and undergraduate students accounted for 16% of voters. The remaining 10% of participants were other professionals, such as research and field technicians or recreational cavers.

During Survey #2, participants suggested 28 additional questions; three questions were duplicates and were thus excluded. The remaining 25 questions were evaluated during Survey #3, and three made it to the 50 top-priority list. The lower threshold for questions was 67% of 'major importance' votes, whereas the top-voted question garnered 91% votes (Fig. 1).

In the following, we present the 50 top-priority questions in subterranean biology according to the results of Surveys #2 and #3 (the full list of questions is provided as online supporting information in Appendix S1). For clarity, questions were compiled into our six subject areas. We provide information about each question's final rank (#) and percentage of 'major importance' votes received (%), and highlight the three questions suggested by the

Survey #2 participants with an asterisk (*). A glossary of terms is available in Table 2.

IV. ADAPTATION

Q1 – What are the drivers of adaptive evolution in caves? [#1; 91%]

Q2 – What are the main constraints to subterranean adaptation? [#4; 83%]

Q3 – What are the degrees of adaptive plasticity of organisms across different subterranean environments? [#9; 78%]

Q4 – Which traits of subterranean organisms should be considered as ‘adaptive’? [#11; 78%]

Q5 – How have morphological and behavioural traits co-evolved in subterranean organisms? [#14; 76%]

Q6 – What is the level and nature of reproductive isolation between cave and surface populations and what reproductive barriers are typically involved? [#19; 75%]

Q7 – Do similar traits evolve repeatedly in subterranean organisms due to changes in the same genes, genetic pathways, and/or developmental processes? [#23; 73%]

Q8 – Have subterranean species evolved a distinct set of convergent behaviours? [#26; 72%]

Q9 – Are there common developmental pathways that promote or constrain subterranean adaptation? [#29; 72%]

Q10 – Do traits that constitute reproductive isolation evolve in the same way across independent closely related subterranean populations or species? [#42; 70%]

The morphology of subterranean organisms, which show bizarre convergent adaptations even across different animal phyla, has historically attracted the attention of generations of scientists (Juan *et al.*, 2010) including Charles Darwin (1859). Therefore, it is no surprise that subterranean biologists participating in this survey greatly valued the role of subterranean habitats as natural laboratories for the study of adaptive evolution. Ten questions focusing on adaptation were included in our top-50 list (Fig. 1).

Colonization of suitable habitat is the initial event leading to subterranean adaptation (details in Section V). Whatever the mode or pathway, colonizers often experience a significant change upon entering the subterranean environment (i.e. complete darkness), which results in visual sensory deprivation, challenges in locating mates and food, limited or modified food resources, and physical barriers to dispersal. Adaptive responses to these factors may involve the action of selection on plastic traits already existing in the colonizers (i.e. phenotypic plasticity; Bilandžija *et al*., 2020), standing genetic variation, or new beneficial mutations. Understanding which of these environmental factors and adaptive responses play a primary role in subterranean adaptation, either acting alone or in various combinations, was the most important question (Q1) in our survey, selected by 91% of participants. Yet, given that some higher taxa are missing or understudied in caves (Culver & Pipan, 2019), it remains unclear what are the main constraints to subterranean adaptation (Q2) and whether specific exaptations facilitate successful colonization events (see also Q11 in Section V). Resolving how many phenotypes of subterranean dwellers depend on genetic and developmental constraints (Q9), or reflect entrapment at local peaks in adaptive landscapes or recent invasions with insufficient time for selection to alter traits, is one of the future challenges for evolutionary biologists.

Additional high-priority questions were focused on subsequent refinements of the initial adaptive responses, such as the repertoire of adaptive plasticity (Q3), the degree to which pre-existing genetic variation contributes to subterranean phenotypes, and which traits of subterranean organisms can be considered as adaptive (Q4). Historically, reduction or loss of traits such as eyes and pigmentation was thought to be driven by random mutations and genetic drift or by natural selection, either directly or indirectly. This controversy has continued to the present, with strong adaptationist (Carlini & Fong, 2017) and non-adaptationist (Wilkens & Strecker, 2017) viewpoints. Depending on the species or ecological context, it is possible that all of these mechanisms have roles in subterranean adaptation. Resolving this debate will require explanations at the molecular, cellular, and developmental levels in multiple lineages (Jeffery, 2005), and the integration of this information to infer whether convergent traits evolve repeatedly in subterranean animals due to changes in the same or different genes, genetic pathways, and developmental processes (Q7). Answers to all these questions will contribute to our understanding concerning why some species adapt rapidly and evolve when facing new environmental conditions, inside or outside caves, which is a critical question given global climate change (Walther *et al.*, 2002). In turn, this could provide insights about adaptive processes occurring in other ecological settings with a similar set of environmental conditions (e.g. permanent darkness, constancy in climatic conditions, food scarcity), such as deep-sea habitats (Trontelj, Borko & Delić, 2019; Mammola, 2020).

Once survival in a subterranean habitat is ensured, the successful colonizers are subject to adaptive morphological and behavioural (co-)evolution (Q5). Many behavioural changes are probably influenced by the essential requirements of finding food and mates in darkness, and may be convergent across different subterranean lineages (Q8). Also, some subterranean animals suddenly attain a new status at the top trophic level and predator release occurs. For example, in the Mexican tetra, *Astyanax mexicanus* (De Filippi) (Actinopterygii: Characidae), the workhorse of adaptive evolution studies in caves (Jeffery, 2009; Wilkens & Strecker, 2017; Torres-Paz *et al.*, 2018), this new ecological status of an apex predator facilitated the evolution of a range of behaviours that may not be sustainable in a predator-limited surface environment (Yoshizawa *et al.*, 2010; Hyacinthe, Attia & Rétaux, 2019).

Most subterranean organisms may also face subsequent invasions of their habitats by new colonizers, of both former surface-dwelling conspecifics (if they are still extant) and other competing species (e.g. Howarth *et al.*, 2007; Wynne *et al.*, 2014). Therefore, to understand subterranean adaptations fully, it is crucial to explore the degree and nature of reproductive isolation between the subterranean-adapted lineages and invading surface conspecifics (Q6). The majority of subterranean animals probably arose through the process of ecological speciation in which reproductive isolation evolved as a response to divergent selection between environments (Niemiller, Fitzpatrick & Miller, 2008; Mammola *et al.*, 2018). Thus, many subterranean adaptations should at least indirectly favour non-random mating between individuals of the derived subterranean and ancestral surface populations. Understanding this will help to address whether traits that constitute reproductive isolation evolve in the same way in independent closely related subterranean populations or species (Q10), and therefore whether and how often parallel speciation occurs in the subterranean realm. Ultimately, this would shed new light concerning the intriguing hypothesis on the predictability of evolution (Blount, Lenski & Losos, 2018).

V. ORIGIN AND EVOLUTION

Q11 – Which traits present in surface species (exaptations) facilitate successful subterranean colonization and adaptation? [#12; 77%]

Q12 – How do, and which, patterns of subterranean species diversification vary across taxa and habitats? [#13; 77%]

Q13 – What evolutionary processes most commonly triggered radiations of subterranean organisms? [#15; 76%]

Q14 – Do subterranean organisms lack genetic variation and thus the ability to adapt to a changing environment? [#16; 75%]

Q15 – Does the timeline of subterranean evolution differ among taxa, types of subterranean habitats, different biogeographic areas, and different ecological settings? [#22; 74%]

Q16 – What are the impact(s) of biotic and abiotic factors on speciation? [#28; 72%]

Q17 – Why are some lineages successful at colonizing subterranean habitats while others are not? [#35; 71%]

Q18 – How old are subterranean species? [#36; 71%]

Q19 – The role of evolutionary processes (convergence/divergence/evolutionary stasis/parallelisms) in subterranean organisms: what are the most common evolutionary processes? [#40; 70%]

Q20 – Are shallow subterranean habitats a gateway to colonize deep zones and is the evolution of deep subterranean species conditioned with a colonization of shallow and later deeper zones? [#41; 70%]

Q21 – What is the rate of evolution of different subterranean traits and does the degree of subterranean adaptation correlate with duration of subterranean inhabitation? [#44; 69%]

Subterranean animals have long interested biologists as evolutionary models. Studies of these species have endeavoured to improve our understanding of evolution, its repeatability at the phenotypic (Friedrich, 2013; Porter & Sumner-Rooney, 2018), physiological (Jones, Cooper & Seymour, 2019), and molecular level (Leys *et al.*, 2005; Bilandžija, Ćetković & Jeffery, 2012;

Niemiller *et al.*, 2013), its reversibility (Copilaş-Ciocianu *et al.*, 2018), and the role of drift in morphological changes (Martínez *et al.*, 2017; Wilkens, 2020). The eleven questions identified highlight how, despite advances in the application of genetic tools and techniques in the last 50 years, fundamental questions regarding the origin and evolution of subterranean animals remain unanswered.

Two high-ranked questions (Q11 and Q17) focused on the traits that enable species to successfully colonize and adapt to subterranean habitats. Additional questions focused on the most common evolutionary processes (Q19), and the influence of biotic and abiotic factors (Q16) that lead to different patterns of diversification across subterranean lineages (Q12). Important subterranean radiations are known in all major taxonomic groups (Deharveng & Bedos, 2019), but only a few of them have been well documented. These include Amphipoda (Zakšek *et al.*, 2019), Collembola (Lukić *et al.*, 2019), and Coleoptera (Leys *et al.*, 2003; Faille *et al.*, 2010; Njunjić *et al.*, 2018). Which evolutionary processes best explain these radiations remains highly debated (Q13) and it would be particularly interesting to compare and contrast radiations of surface-dwelling plants and animals (Gillespie *et al.*, 2020) with subterranean-adapted species to determine if any universal patterns exist. For many animal groups, subterranean species are commonly assumed to have evolved from surface species (Barr & Holsinger, 1985; Peck & Finston, 1993), but recent phylogenetic studies suggest that this assumption may not always apply (Faille *et al.*, 2010; Juan *et al.*, 2010; Leijs *et al.*, 2012). Speciation and diversification may also occur within the confines of a subterranean habitat, a process referred to as 'endogenous diversification' (Trontelj, 2019). Moreover, some phylogenetic studies suggested that subterranean colonization is not an evolutionary dead end and surface species may actually arise from subterranean ancestors (Prendini, Francke & Vignoli, 2010; Niemiller *et al.*, 2013; Copilaş-

Ciocianu *et al.*, 2018). However, cases of endogenous speciation and ‘subterranean to surface’ reversals are potentially confounded by extinction of surface lineages (Juan *et al.*, 2010). Therefore, new approaches are needed that avoid reliance on phylogenetic methods alone to improve our understanding of these patterns.

Genetic variation enhances the ability of species to adapt and diversify. Additionally, it has been shown that some subterranean species may contain high levels of neutral genetic variation (Buhay & Crandall, 2005; Guzik *et al.*, 2009), but it is still unclear whether neutral mutations equates to high levels of adaptive genetic variation. This underpins the question whether subterranean species lack the ability to adapt to changing environments (Q14), including increasing temperatures and the introduction of new pathogens (Mammola *et al.*, 2019*c*). Such hypotheses are obviously not exclusive to the subterranean environment. However, this ecosystem does provide numerous examples of how low genetic variation was hypothesized to be related to low adaptive capacity, a phenomenon more common underground than at the surface (Konec *et al.*, 2015; Lefébure *et al.*, 2017; Fumey *et al.*, 2018).

Understanding the timeline and direction of subterranean evolution, as well as the age of subterranean species, featured prominently in several questions (Q15, Q18, Q20, Q21). Advances in molecular clock calibration (Drummond *et al.*, 2006) and genomic analyses (Pérez-Moreno *et al.*, 2016) are considerably promising and permit the development of robust time trees (Pons *et al.*, 2019). However, these analyses are limited by the availability of extant and fossil taxa and the extinction of surface relatives; the latter makes it difficult to pinpoint the initial colonization time of a subterranean habitat by a given species. This is particularly important for ancient lineages of specialized subterranean organisms with marine origin, which often lack surface-dwelling relatives and/or show low levels of fossilization (Pérez-Moreno *et al.*, 2016).

This is unfortunate because many of these basally branching lineages are required to reconstruct trait evolution of major animal lineages (e.g. Johnson *et al.*, 2012; Khodami *et al.*, 2017; Lozano-Fernandez *et al.*, 2019).

The genetic basis underlying evolution of subterranean traits, and how they are shaped by natural selection and/or neutral processes, are key factors in determining rates of subterranean evolution (Q21). Considerable advances have been made through the study of model subterranean species, especially *Astyanax mexicanus* and the freshwater isopod *Asellus aquaticus* (L.) (Protas & Jeffery, 2012). These species have several independent and recently evolved subterranean populations, as well as extant surface populations, which can be hybridized in the laboratory. Their features allow for the dissection of genes and mutations responsible for traits related to subterranean life and provide information on the processes (e.g. selection or neutral evolution) that shape their evolution. The role of neutral processes in the evolution of subterranean animals has also been explored using alternative model systems (e.g. dytiscid beetles and amblyopsid cavefishes). In both cases, species have been evolving underground for millions of years, which is sufficient to enable the fixation of deleterious mutations in genes under relaxed selection (Niemiller *et al.*, 2013; Tierney *et al.*, 2018). These model organisms offer great potential to investigate major questions on the origin and evolution of subterranean animals using comparative genomics, and thus may provide insights for similar processes in other, non-subterranean, settings.

VI. COMMUNITY ECOLOGY

Q22 – What are the main ecological and ecosystem services provided by subterranean populations and communities? [#20; 75%]

Q23 – What are the key food-web processes influencing subterranean community dynamics? [#24; 73%]

Q24 – How do stochastic events interact with long-term trends in subterranean ecosystems? [#30; 72%]

Q25 – How do basic life-history characteristics differ among subterranean communities and between subterranean and surface communities? [#33; 71%]

Subterranean habitats are well-suited systems to address general problems in community ecology (Mammola, 2019). Foremost, caves are often semi-closed environments extensively replicated across the Earth (Culver, 1970; Culver & Pipan, 2019; Itescu, 2019; Mammola, 2019). Second, subterranean communities generally exhibit lower diversity and abundance of organisms than surface ones and are characterized by a bottom-truncated functional diversity (Gibert & Deharveng, 2002), allowing us to disentangle the effect of abiotic conditions and biotic interactions in filtering species possessing specific traits within the community (Cardoso, 2012). Third, caves have some conspicuous environmental gradients from the surface towards the subsurface (Howarth, 1982; Tobin, Hutchins & Schwartz, 2013; Mammola *et al.*, 2019*d*), offering a mosaic structure of subterranean microhabitats defined by distinct habitat-filtering properties (Trontelj, Blejec & Fišer, 2012; Mammola *et al.*, 2020).

Four questions in community ecology made it to the top-50 list. This result reflects a general trend in subterranean biology, where researchers have primarily focused on caves as model systems for evolutionary studies (Juan *et al.*, 2010), and secondarily used caves as convenient settings to address fundamental ecological questions (Mammola, 2019). Yet, these four questions fell within general and timely areas of current ecological research (see Sutherland *et al.*, 2013).

The top-ranked question underscored the importance of services provided to humans by subterranean species and ecosystems (Q22), rather than on theoretical aspects of community ecology. Examples of ecosystem services provided by subterranean ecosystems include pollination, seed dispersal, and agricultural pest control by bats (Kunz *et al.*, 2011; Medellin, Wiederholt & Lopez-Hoffman, 2017), provision of clean water (Griebler & Avramov, 2015), serving as a source for new pharmaceutical products (Cheeptham *et al.*, 2013), and even cheese production (Ozturkoglu-Budak *et al.*, 2016). While services with direct benefit to humans have received some attention, values provided by subterranean ecosystems extend far beyond direct human needs. In light of emerging conservation issues associated with subterranean ecosystems (Mammola *et al.*, 2019*b*), investigating ecological services and links between above- and below-ground diversity in ecosystem functioning is crucial.

Two questions called for more research into life-history characteristics (e.g. growth rates, age and size at sexual maturity, longevity, and survival rates; Q25) and food-web specificities of subterranean communities (Q23). Interactions among life-history traits determine the fitness of each population, while interactions between populations and the environment dictate the distribution of species (Steranrs, 1992). Only a few studies have described life histories of subterranean species, and this is partially explained by the challenges of captive breeding and the technical problems and effort necessary to conduct *in situ* comprehensive studies (Vonk & Nijman, 2006; Voituron *et al.*, 2011; Venarsky, Huryn & Benstead, 2012; Riesch *et al.*, 2016;

Simon *et al.*, 2017). Consequently, the lack of knowledge on cave species traits limits our understanding of evolutionary and ecological processes occurring in subterranean ecosystems.

Energy limitation is considered a primary mechanism influencing both evolutionary and ecological processes in subterranean environments (Venarsky & Huntsman, 2018). However, a more nuanced understanding of subterranean food-web dynamics (Q23) will require other research actions, including to (*i*) understand the spatial and temporal dynamics of energy resources; (*ii*) compare resource quality with consumers' physiological requirements; and (*iii*) compare consumption rates with resource availability in subterranean habitats with different environmental conditions (e.g. terrestrial *versus* aquatic, fresh *versus* salt water, and detrital *versus* chemolithoautotrophic food webs).

Finally, understanding the role of stochastic events in caves was highlighted as a deficient area in community ecology (Q24). Given that these events are increasing in frequency amid the environmental crisis of the new millennium (Rahmstorf & Coumou, 2011), the study of stochastic phenomena has emerged as a central topic in ecology (Scheffer *et al.*, 2001). Recent papers used groundwater crustaceans to elucidate some of the mechanisms by which earthquakes affect the composition and structure of biological communities (Galassi *et al.*, 2014; Fattorini *et al.*, 2017; Fattorini, Di Lorenzo & Galassi, 2018; Morimura *et al.*, 2020). Additional studies have focused on the effect of other events, such as heavy precipitation (Calderón-Gutiérrez, Sánchez-Ortiz & Huato-Soberanis, 2018) and flooding (Pacioglu *et al.*, 2019). Although it may seem counterintuitive to study stochastic environmental shifts in caves, as they have been traditionally perceived as stable ecosystems, these examples show how caves may represent promising model systems for quantifying the impacts of abrupt environmental shifts driving ecosystem evolution (Mammola, 2019).

VII. MACROECOLOGY AND BIOGEOGRAPHY

Q26 – What drives subterranean patterns of phylogenetic and functional diversity? [#21; 75%]

Q27 – Would the use of novel molecular methods (e.g. metabarcoding, environmental DNA) provide new insights on subterranean biodiversity patterns and affect known patterns? [#27; 72%]

Q28 – What is the species richness pattern of subterranean organisms globally? [#31; 72%]

Q29 – What factors drive the relative importance of speciation, extinction, and dispersal in shaping subterranean diversity patterns across regions? [#34; 71%]

Q30 – Are current subterranean biodiversity patterns best explained by history of colonization of surface ancestors or by *in situ* speciation and dispersal in subterranean habitats? [#39; 70%]

Q31 – How can sampling effort be standardized so that comparisons of species richness are unbiased? [#43; 69%]

Over the last 20 years, research in subterranean ecology is shifting from local to landscape studies aiming to document and understand biodiversity patterns at regional to global scales (Zagmajster *et al*., 2019). This transition is not without difficulties, as it requires linking biodiversity patterns to eco-evolutionary processes with little to no possibility for manipulative experiments. Six questions in 'Macroecology and biogeography' were identified in the top-50 list (Fig. 1). These questions mirror the main challenges faced when documenting and understanding broad-scale biodiversity patterns at the surface. The first challenge is assembling the data required to bring out the characteristic features of biodiversity patterns at such broad scales, while ensuring these patterns are not biased by sampling effort (Q28, Q31). Secondly, to combine multiple sampling techniques, species identification methods (e.g. morphological and

DNA-based identification), and biodiversity metrics (e.g. alpha, beta, and gamma diversity) in a meaningful way to elucidate the many facets of biodiversity patterns (e.g. taxonomic, phylogenetic, and/or functional diversity; Jarzyna & Jetz, 2016) (Q27, Q26). Lastly, the relative contributions of different evolutionary processes (Q29) and diversification hypotheses (Q30) in shaping biodiversity patterns should be fully examined.

The publication of global subterranean diversity maps and databases is a recent phenomenon (Culver & Pipan, 2019; Zagmajster *et al.*, 2019). While diversity maps are informative as they portray differences in species richness among regions or countries, we still lack global maps showing species richness for spatial units of equal area [but see Zagmajster, Culver & Sket (2008), Niemiller & Zigler (2013), and Eme *et al.* (2015) for examples of regional- and continental-scale diversity maps]. Several approaches have been developed to minimize differences in species richness due to sampling bias (Q31). This issue is particularly germane to difficulties in sampling subterranean habitats. For example, sampling protocols were typically standardized among sites and completeness of species inventories were assessed using accumulation and rarefaction curves (Zagmajster *et al.*, 2008; Dole-Olivier *et al.*, 2009; Wynne *et al.*, 2018). Also, observed species richness patterns were tested for robustness using species richness estimators (Zagmajster *et al.*, 2014), or complemented with species richness predictions modelled from environmental data (Mokany *et al.*, 2019).

Beyond accounting for sampling biases, molecular methods are increasingly useful in understanding subterranean biodiversity patterns (Q27). For example, a recent study comparing latitudinal patterns of crustacean species range size obtained from morphology- and DNA-based species delimitation showed that the pattern of increasing median range size at higher latitudes was more evident when delimiting species with DNA (Eme *et al.*, 2018) (Fig. 2). As sequencing becomes increasingly applied to subterranean taxa, environmental DNA sampling and monitoring may be also used to detect these species in areas difficult to access (Gorički *et al.*, 2017; Niemiller *et al.*, 2018), thus resulting in more accurate maps of their distributions. To our knowledge, patterns of phylogenetic and functional diversity at continental to global scales have not been documented for any subterranean taxon (Q26), despite the growing knowledge of phylogenetic relationships and species traits (Morvan *et al.*, 2013; Fernandes, Batalha & Bichuette, 2016; Fišer *et al.*, 2019; Mammola *et al.*, 2020). Documenting these patterns will further underscore the relative importance of dispersal, extinction, and different speciation modes in shaping geographic variation of species richness. Given the differences in global diversity patterns between subterranean and surface habitats, comparing the two systems might help further to elucidate the key drivers of diversity.

Recent macroecological studies have shown that historical climatic variability, spatial heterogeneity, and energy contribute to species richness patterns of subterranean taxa in Europe. However, the contributions of these factors vary regionally and across taxa (Eme *et al.*, 2015; Bregović & Zagmajster, 2016; Bregović, Fišer & Zagmajster, 2019; Mammola *et al.*, 2019*a*). At a landscape scale, linking environmental factors with speciation, extinction, and dispersal dynamics (Q29), as well as diversification processes (Q30), remains challenging and requires the use of phylogenetic methods and a large number of specimens for DNA analysis (Stern *et al.*, 2017). Yet phylogenetic methods encompass uncertainties that are highly sensitive to sampling bias and the confounding effect of extinction, both obscuring the inference of transitions to subterranean life. To ameliorate this, genes that lose their function soon after the transition should be used (Lefébure *et al.*, 2017) (see also Section V).

VIII. CONSERVATION

Q32 – How does climate change affect subterranean-adapted organisms? [#2; 84%]

Q33 – What are the effects of pollution on subterranean-restricted microorganisms, arthropods, and vertebrates? [#3; 84%]

Q34 – What is the impact of above-ground disturbance on subterranean environments and their fauna? [#5; 82%]

Q35 – How can we evaluate the ecological status of subterranean ecosystems? [#6; 80%]

Q36 – How can we protect subterranean-adapted species from invasive species? [#7; 80%]

Q37 – How can we combine policy, education, research, and management to safeguard subterranean biodiversity effectively? [#8; 80%]

Q38* – What factors determine the size and location of effective protected areas in subterranean environments? [#10; 78%]

Q39* – How can we effectively involve governments and key stakeholders in the conservation of caves and other subterranean systems? [#17; 75%]

Q40 – What would be the best monitoring protocols to quantify long-term changes in the distribution and abundance of subterranean invertebrates? [#18; 75%]

Q41 – How do we address the lack of knowledge (biodiversity shortfalls) about the biology of subterranean species to enhance proper conservation measures? [#25; 73%]

Q42 – Can subterranean-adapted organisms be used as bioindicators of the health of subterranean ecosystems? [#45; 69%]

Q43 – How does the use of caves by humans (e.g. tourism, religious, therapeutic, and recreational activities) affect subterranean ecosystems? [#48; 68%]

Ecosystems are experiencing biodiversity loss at an unprecedented rate worldwide (Barnosky *et al.*, 2011; Dirzo *et al.*, 2014; IPBES, 2018; Cardoso *et al.*, 2020). Thus, conservation and management of cave biological diversity is of the utmost concern among subterranean biologists (Mammola *et al.*, 2019*b*). Conservation questions comprised most of the questions (24%) in our top-50 list (Fig. 1). Of these, 10 questions were part of the initial List #1, while two additional questions were suggested by survey participants. Three questions (Q32, Q33, and Q36) highlighted three of the greatest threats to biodiversity worldwide – climate change (Ripple *et al.*, 2019), pollution (Ripple *et al.*, 2017), and invasive alien species (Pyšek *et al.*, 2020) – whose effects are pervasive also underground (Mammola *et al.*, 2019*b*). Additional questions were centred on the impacts of above-ground disturbance (Q34) and human activities (Q43) on subterranean habitats. All these threats can be combined and described as 'habitat loss and degradation', which is one of the most important drivers of biodiversity loss globally (IPBES, 2018). Subterranean habitat loss and degradation is primarily due to surface activities, such as agricultural expansion and intensification, urbanization, and mining activities (Reboleira *et al.*, 2013; Mammola *et al.*, 2019*b*; Castaño-Sánchez, Hose & Reboleira, 2020). Human activities inside caves may also constitute localized threats, with recreational use and tourism activities being of particular concern (Fernandez-Cortes *et al.*, 2011; Faille, Bourdeau & Deharveng, 2015). In certain areas, people are even poaching rare invertebrate species for private collections (Simičević, 2017), as in the discussed case of *Anophthalmus hitleri* Scheibel (Coleoptera: Carabidae) (Berenbaum, 2010).

Evaluating, understanding, and mitigating these threats are primarily hampered by our scarce knowledge of subterranean organisms' biology (Q41), especially life-history traits (see Q25 in Section VI). Understanding changes in species' abundance and distribution will be crucial to halting biodiversity loss in subterranean habitats. Studies aimed at identifying bioindicator species (Q42) to help bolster long-term monitoring programs (Q40) are needed. Additionally, improved sampling procedures and characterizing cave communities in previously undocumented areas would both enhance our knowledge of subterranean biodiversity (Mammola *et al.*, 2019*b*) and improve the effectiveness of conservation measures (Q41).

Furthermore, it is crucial to adopt innovative approaches to safeguard subterranean biodiversity (Q37), as well as to determine the size and location of effective protected areas (Q38). Standardized systematic sampling techniques have been applied to terrestrial (Wynne *et al.*, 2018, 2019) and aquatic subterranean invertebrate species (Dole-Olivier *et al.*, 2009); to be optimally beneficial to conservation and monitoring, these techniques will need to be further scrutinized across a large breadth of taxa and systems. Recently, a cave vulnerability assessment protocol has been developed for bat cave roosts (Tanalgo, Tabora & Hughes, 2018) and, if refined, would hold promise for use with other subterranean animals.

Protected areas are the most crucial measure to safeguard specific subterranean habitats and the sensitive animal populations they often support (Q38). Indices have been developed for site selection and conservation prioritization (e.g. Borges *et al.*, 2012; Rabelo, Souza-Silva & Ferreira, 2018; Strona *et al.*, 2019; Fattorini *et al.*, 2020) which are often based on complementarity, flexibility, and irreplaceability principles (Michel *et al.*, 2009). Yet, rigorous geospatial analysis is still rarely applied when the extents of protected areas are being determined. Further considerations should include managing lands upslope from caves or entire watersheds supporting sensitive subterranean habitats. If a species-level approach is taken for establishing a protected area, it would be reasonable to protect the land at the hydrogeologic unit (i.e. watershed or karst/volcanic unit) level – as animals are expected to use mesocaverns or unconsolidated sediments for dispersal (Howarth, 1983; Malard *et al.*, 2017; Trontelj, 2019). Importantly, such an approach should be based on the most accurate estimation of the relevant animal's distributional range.

While effective legislation and/or management plans exist for some subterranean species and some regions of the world, overall management policies for most regions of speleological importance are lacking (Q39). Only a few countries have national cave protection laws. For example, the United States Federal Cave Protection Act of 1988 has been used as a tool to manage caves on federally owned lands, while Brazil requires geological and biological assessments of caves and stipulates mitigation of any human activities that may negatively impact cave natural resources. In any case, to be fully operational, such legislative and management tools need to be based on the best available science including a comprehensive knowledge of fauna distribution (Brooks, Da Fonseca & Rodrigues, 2004; Samways *et al.*, 2020) and traits of the species of concern (Chichorro, Juslén & Cardoso, 2019; Fattorini *et al.*, 2020). Importantly, management plans will require both financial, governmental, and local community support for their implementation. Unfortunately, most countries lack the capacity or legislation to protect and conserve sensitive subterranean resources.

IX. MICROBIOLOGY AND APPLIED TOPICS

Q44 – What is the role of Bacteria, Archaea, fungi, and viruses in nutrient cycling in subterranean systems? [#32; 71%]

Q45 – How adaptable are cave microorganisms to changing environmental conditions (e.g. climate change)? [#37; 70%]

Q46 – How do other organisms (humans and other animals), and their activities (e.g. visiting humans and global climate change) influence cave microbiome diversity patterns? [#38; 70%]

Q47 – How does the range of energy sources and quantity influence the diversity of subterranean microbiota? [#46; 68%]

Q48 – What are the limiting nutrients for subterranean microbiota and how do they affect overall subterranean microbial diversity? [#47; 68%]

Q49 – How do subterranean microorganisms cycle key elements – nitrogen, iron, carbon, sulfur, and phosphorus? [#49; 67%]

Q50* – What is the role of microorganisms in cave-formation processes (speleogenesis) in subterranean environments? [#50; 67%]

Without a doubt, topics such as adaptation, origin and evolution, community dynamics, and biogeographic distribution patterns are similarly important and actively targeted in microbial ecology (Antwis *et al.*, 2017). However, research in macroecology and microbial ecology is often conducted separately rather than hand-in-hand. For nearly 200 years, subterranean ecosystems have been studied from a macroscopic perspective. Subterranean microbiological research is a relatively new discipline with most research having been conducted since the middle of the last century (Griebler & Lueders, 2009). A modern ecosystem approach to subterranean biota requires consideration across all trophic levels and scales (Hershey & Barton, 2019), especially since the 1980s, when the first cave ecosystems fully sustained by *in situ* chemosynthetic primary production were discovered (Sarbu, Kane & Kinkle, 1996; Kumaresan *et al.*, 2014).

The seven questions on the top-50 list address general problems that have been frequently examined for various subterranean ecosystems, such as alluvial aquifers, however, less systematically for cave environments. Three questions focused on the active role of microorganisms in nutrient cycling (Q44, Q49) and how nutrient limitations influence microbial diversity (Q48). Although we know that microbes rule the subsurface in terms of element cycles (Ortiz *et al.*, 2014; Kimble *et al.*, 2018) and constitute the basis of the food web, we still lack detailed information on conversion rates and growth kinetics. In addition, subterranean organisms often persist with limited energy resources. Thus, understanding their specific adaptations would help advance our understanding of adaptive strategies for microorganisms in other ecosystems (e.g. mountain-summit and deep-sea habitats). Additionally, the role of viruses, which only recently has been recognized as 'tremendous' for groundwater ecosystems (Griebler, Malard & Lefébure, 2014), has not been investigated for terrestrial subterranean systems (Q44).

Two questions further addressed the resistance and resilience of cave microbial communities to disturbance from changes in environmental conditions (Q45) (Cavicchioli *et al.*, 2019), and the impacts of other organisms (in particular, humans; Moldovan *et al.*, 2020; Martínez *et al*., 2020) on microbial diversity (Q46). These questions also were related to conservation issues from a microbiological perspective. The adverse impacts of the fungus *Pseudogymnoascus destructans* that causes white-nose syndrome in North American bats is a prominent example. To date, *P. destructans* occurs in 38 U.S. states and seven Canadian provinces (see http://www.whitenosesyndrome.org), which raises serious concerns for the conservation of hibernating bat species and the ecosystem services they provide (Kunz *et al.*, 2011; Boyles *et al.*, 2011; Medellin *et al.*, 2017; Mammola *et al.*, 2019*b*). The fungus is an opportunistic environmental pathogen, which can remain in the subterranean environment and contribute to the cave microbiome even in the absence of its host (Lorch *et al.*, 2013).

It has been hypothesized that microbial communities with high diversity and functional redundancy do not select for ecosystems poor in energy and stable in environmental conditions (Griebler & Lueders, 2009). Thus, the introduction of novel species may have a destabilizing effect on a cave's biological equilibrium (Q46). The same is true for the introduction of contaminants, such as organic compounds and nutrients that provide additional energy. We are only beginning to understand whether and how energy–diversity relationships known from macroecology apply to complex natural bacterial communities (Q47). In fact, there is a growing body of evidence that diversity–productivity relationships also drive microbial communities (Smith, 2007), but this question has not been examined systematically in subterranean ecosystems yet.

Finally, Q50 points to the potential contribution of microorganisms in speleogenetic processes, such as weathering and rock formation *via* inducing precipitation. Specifically, in terms of (inorganic) carbon cycling in face of climate change, the role of microbes in the formation of caves may be of great relevance, and has yet to be fully examined.

X. CONCLUSIONS

(1) The 50th anniversary of Poulson & White's (1969) article was the perfect time to reflect on milestone scientific achievements obtained in the natural laboratories offered by caves, while also delineating the most important research priorities for years to come. We have shown how subterranean biology has contributed strongly to general scientific questions *via* the study of evolutionary and ecological processes along the vertical dimension (i.e. the evolutionary transition from the surface to the subsurface). These accomplishments resonate with the sentiments of Poulson & White (1969) and we anticipate that biologists will continue to unravel the mysteries of subterranean ecosystems and contribute to scientific knowledge more broadly,

insofar as revolutionary advances in approaches and technologies continue to foster and nurture novel paradigms.

(2) There is a significant lack of knowledge concerning eco-evolutionary processes underlying biodiversity patterns along the horizontal gradient (i.e. within subterranean habitats). This is largely driven by a paucity of functional ecology studies, the weakness of trait-based approaches (Cardoso, 2012; Fernandes *et al.*, 2016; Fišer *et al.*, 2019; Mammola *et al.*, 2020), and the lack of robust systematic sampling techniques for most taxonomic groups (Wynne *et al.*, 2019). Bridging these gaps will significantly influence how we address and prioritize future research on the conservation and ecosystem services of subterranean habitats (e.g. Fattorini *et al.*, 2020), as emphasized by the large number of unresolved questions in conservation biology (representing nearly 25% of the top-50 list).

(3) We also invite scientists to redouble their efforts to understand the diversity of subterranean life across all its components, with a special focus on linking macroscopic and microbial ecology (Foulquier *et al.*, 2011; Mermillod-Blondin, 2011). This will enable us to achieve a mechanistic understanding of subterranean eco-evolutionary processes and ecosystem function. This information will be critical in guiding future policy decisions as human activities and global environmental change increasingly impact and strain the subterranean realm.

(4) There is a concern that simple voting exercises such as this one may favour general over specific questions. Perhaps as a result of this bias, some of the top-voted questions appear to be broad in scope (e.g. Q1, Q2, and Q32). While these questions were able to capture important general lines of inquiry, specific questions may be more useful for setting applied agendas. Therefore, we invite interested readers to consult Appendix S1, which contains our complete list of 120 questions.

(5) While the ‘caves as laboratory’ paradigm is an effective way to frame broadly scoped studies, we recognize the top-50 list of questions primarily pertains to unresolved issues within the borders of subterranean biology. Yet subterranean habitats offer much more. Deep subterranean habitats are one of the few natural systems defined by highly stable and homogenous climatic conditions tantamount to those maintained in a laboratory (Sánchez-Fernández *et al.*, 2018). These systems have an island-like nature (Itescu, 2019), and often support communities characterized by highly specialized organisms interacting in simplified ecological networks (Mammola, 2019). By extension, a robust understanding of these rather simplified settings may enable researchers to disentangle the complexities of more diverse systems (e.g. deep-sea habitats).

(6) Ultimately, all these features point at subterranean ecosystems as ideal settings in which to tackle general questions. We strived to provide examples of how some of our survey questions may aid in addressing non-cave specific agendas. Our hope is that this horizon scan exercise both underscores the importance of caves for addressing a range of eco-evolutionary questions, as well as stimulates researchers to redouble their efforts to address some of these lingering questions in subterranean biology.

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XIII. SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Questions from List #2 (i.e. 120 questions selected from List #1 during Survey#1) and List #3 (i.e. 25 additional questions suggested by Survey #2 participants) ranked based on the percentage of ‘major importance’ votes.

Table 1. Subject areas, general topics addressed, panel member composition (*= panel coordinator; °= postdoc or early career researcher), and number of questions included in the top-50 list out of the total retained in List #1. Panel members are listed alphabetically by surname.

Subject area	**General topics**	**Panel members**	**Number of questions**
Adaptation	Morphological, physiological and behavioural adaptations to the subterranean environment	Žiga Fišer°, Daniel W. Fong, Tanja Pipan*, William R. Jeffery, Jure Jugovic	10 out of 43
Origin and evolution	Cave ontology and past climate change, migration–speciation–extinction dynamics, and speciation and diversification	Steven J.B. Cooper*, Matthew Niemiller, Alejandro Martínez°, Meredith Protas	11 out of 36
Community ecology	Population dynamics, community assembly, biotic interaction, trophic webs, and energy flows	Rodrigo L. Ferreira*, Cene Fišer, Thais G. Pellegrini°, Michael Venarsky°	4 out of 32
Macroecology and biogeography	Global diversity patterns (taxonomic, phylogenetic, functional), biogeography theory, and diversity drivers	Maria E. Bichuette, David Eme°, Florian Malard*, Maja Zagmajster°	6 out of 32
Conservation biology	Climate change, habitat loss, invasive species, conservation and management policies, and show-cave-related issues	Isabel R. Amorim°, Paulo A. V. Borges*, Louis Deharveng, J. Judson Wynne, Ana Sofia P. S. Reboleira	12 out of 37
Microbiology and applied topics	Microbial communities, industrial and pharmaceutical potential, epidemics, and exobiology	Naowarat Cheeptham, Thomas M. Lilley*, Melissa B. Meierhofer°, Diana E. Northup	7 out of 31
Other topics	Any topic falling outside the scope of the six core subject areas	David C. Culver*, Christian Griebler, Johanna Kowalko, Raoul Manenti°	n/a (merged within the other subject areas)

Table 2. Glossary of terms.

Term	**General definition**
Cave	A human-accessible subterranean space, either a single chamber or series of chambers, formed within different substrata (Curl, 1964). Note that a cave is just one among the wide variety of subterranean habitats (see definition below).
Exaptation	A trait shaped by selection or neutral evolution co-opted for a new function (Gould & Vrba, 1982).
Speleogenetic process	The process of water dissolving surrounding rock, gradually forming passages that evolve into cave systems (Audra & Palmer, 2011).
Subterranean habitat(s) / ecosystem(s)	The breadth of underground voids of different sizes, either dry or filled with water, sharing two main ecological features: the absence of sunlight and buffered climatic conditions. Examples of subterranean habitats include caves, groundwater, anchialine systems, artificially excavated underground voids, shallow subterranean habitats, as well as deep maze of fissures and pore spaces with size prohibiting human entry (Culver & Pipan, 2019).

FIGURE LEGENDS

Fig. 1. Survey workflow, summary statistics of survey participants, and the breakdown by subject area of the 50 highest priority research questions.

Fig. 2. The relationship between median range size (maximum linear extent) per latitudinal band and latitude for 147 European groundwater species of Niphargidae (Amphipoda) and Aselloidea (Isopoda) delimited using morphology (A) and a molecular species delimitation method (B). Molecular delimitation was performed by a Bayesian implementation of the Poisson tree processes (Zhang *et al*., 2013) approach based on molecular phylogenies inferred from 2883 cytochrome *c* oxidase subunit I sequences. Black horizontal bars, dots, and boxes show the median, average, and interquartile range, respectively, for 0.9° latitudinal bands. The maximum length of each whisker is up to 1.5 times the interquartile range. Trend lines (with 95% confidence intervals) represent the fit of a gamma generalized linear model to the averages of latitudinal bands and its quadratic (A) and cubic (B) term. Data re-analysed from Eme *et al.* (2018).